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(54) Title: OPTHALMIC COMPOSITIONS FOR TREATING OCULAR HYPERTENSION

(57) Abstract: This invention relates to potent potassium channel blocker compounds of Formula I or a formulation thereof for the treatment of glaucoma and other conditions which leads to elevated intraocular pressure in the eye of a patient. This invention also relates to the use of such compounds to provide a neuroprotective effect to the eye of mammalian species, particularly humans.



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TITLE OF THE INVENTION

OPHTHALMIC COMPOSITIONS FOR TREATING OCULAR HYPERTENSION

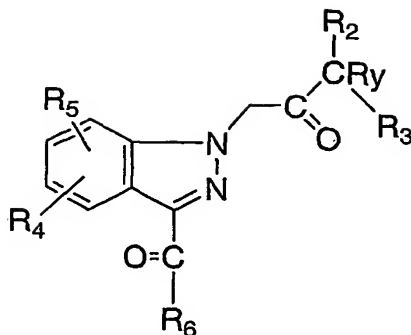
BACKGROUND OF THE INVENTION

5 Glaucoma is a degenerative disease of the eye wherein the intraocular pressure is too high to permit normal eye function. As a result, damage may occur to the optic nerve head and result in irreversible loss of visual function. If untreated, glaucoma may eventually lead to blindness. Ocular hypertension, i.e., the condition of elevated intraocular pressure without optic nerve head damage or characteristic glaucomatous visual field defects, is now believed by the majority of ophthalmologists to represent merely the earliest phase in the onset of glaucoma.

10 There are several therapies for treating glaucoma and elevated intraocular pressure, but the efficacy and the side effect profiles of these agents are not ideal. Recently potassium channel blockers were found to reduce intraocular pressure in the eye and therefore provide yet one more approach to the treatment of ocular hypertension and the degenerative ocular conditions related thereto. Blockage of potassium channels can diminish fluid secretion, and under some circumstances, increase smooth muscle contraction and would be expected to lower IOP and have neuroprotective effects in the eye. (see US Patent Nos. 5,573,758 and 5,925,342; Moore, et al., Invest. Ophthalmol. Vis. Sci 38, 1997; WO 89/10757, WO94/28900, and WO 96/33719).

SUMMARY OF THE INVENTION

20 This invention relates to the use of potent potassium channel blockers or a formulation thereof in the treatment of glaucoma and other conditions which are related to elevated intraocular pressure in the eye of a patient. This invention also relates to the use of such compounds to provide a neuroprotective effect to the eye of mammalian species, particularly humans. More particularly this invention relates to the treatment of glaucoma and/or ocular hypertension (elevated intraocular pressure) using novel indazole compounds having the structural formula I:



Formula I

or a pharmaceutically acceptable salt, enantiomer, diastereomer or mixture thereof:

5 wherein,

R represents hydrogen, or C₁₋₆ alkyl;

RY represents H, or C₁₋₆ alkyl;

10

R_w represents H, C₁₋₆ alkyl, -C(O)C₁₋₆ alkyl, -C(O)OC₁₋₆ alkyl, -SO₂N(R)₂, -SO₂C₁₋₆ alkyl, -SO₂C₆₋₁₀ aryl, NO₂, CN or -C(O)N(R)₂;

15

R₂ represents hydrogen, C₁₋₁₀ alkyl, OH, C₂₋₆ alkenyl, -(CH₂)_nO(CH₂)_mOR, -(CH₂)_nC₁₋₆ alkoxy, -(CH₂)_nC₃₋₈ cycloalkyl, -(CH₂)_nC₃₋₁₀ heterocyclyl, or -(CH₂)_nC₆₋₁₀ aryl, said alkyl, heterocyclyl, or aryl optionally substituted with 1-3 groups selected from R_a;

20

R₃ represents hydrogen, C₁₋₁₀ alkyl, -(CH₂)_nC₃₋₈ cycloalkyl, -(CH₂)_nC₃₋₁₀ heterocyclyl, -(CH₂)_nCOOR, -(CH₂)_nC₆₋₁₀ aryl, nitro, cyano or halogen, said alkyl, heterocyclyl, or aryl optionally substituted with 1-3 groups of R_a;

25

R₄ and R₅ independently represent hydrogen, C₁₋₆ alkoxy, OH, C₁₋₆ alkyl, COOR, SO_qC₁₋₆ alkyl, COC₁₋₆ alkyl, SO₃H, -O(CH₂)_nN(R)₂, -O(CH₂)_nCO₂R, -OPO(OH)₂, CF₃, OCF₃ - N(R)₂, nitro, cyano, C₁₋₆ alkylamino, or halogen; and

R₆ represents hydrogen, C₁₋₁₀ alkyl, -(CH₂)_nC₆₋₁₀ aryl, -(CH₂)_nC₃₋₁₀ heterocyclyl, - (CH₂)_nC₃₋₈ cycloalkyl, said aryl, heterocyclyl and alkyl optionally substituted with 1-3 groups

selected from R^a , wherein the $R^a(s)$ can be attached to any carbon atom or heteroatom selected from N and S;

5 R_8 represents $-(CH_2)_n C_{3-8}$ cycloalkyl, $-(CH_2)_n$ 3-10 heterocyclyl, C_{1-6} alkoxy or $-(CH_2)_n C_{5-10}$ heteroaryl, $-(CH_2)_n C_{6-10}$ aryl said heterocyclyl, aryl or heteroaryl optionally substituted with 1-3 groups selected from R^a ;

10 R^a represents F, Cl, Br, I, CF_3 , $N(R)_2$, NO_2 , CN, -O-, -COR₈, -CONHR₈, -CON(R₈)₂, -O(CH₂)_nCOOR, -NH(CH₂)_nOR, -COOR, -OCF₃, CF_2CH_2OR , -NHCOR, -SO₂R, -SO₂NR₂, -SR, $(C_1-C_6 \text{ alkyl})O-$, $-(CH_2)_n O(CH_2)_m OR$, $-(CH_2)_n C_{1-6}$ alkoxy, (aryl)O-, $-(CH_2)_n OH$, $(C_1-C_6 \text{ alkyl})S(O)_m-$, $H_2N-C(NH)-$, $(C_1-C_6 \text{ alkyl})C(O)-$, $(C_1-C_6 \text{ alkyl})OC(O)NH-$, $-(C_1-C_6 \text{ alkyl})NR_w(CH_2)_n C_{3-10}$ heterocyclyl- R_w , $-(C_1-C_6 \text{ alkyl})O(CH_2)_n C_{3-10}$ heterocyclyl- R_w , $-(C_1-C_6 \text{ alkyl})S(CH_2)_n C_{3-10}$ heterocyclyl- R_w , $-(C_1-C_6 \text{ alkyl})-C_{3-10}$ heterocyclyl- R_w , $-(CH_2)_n-Z^1-C(=Z^2)N(R)_2$, $-(C_{2-6} \text{ alkenyl})NR_w(CH_2)_n C_{3-10}$ heterocyclyl- R_w , $-(C_{2-6} \text{ alkenyl})O(CH_2)_n C_{3-10}$ heterocyclyl- R_w , $-(C_{2-6} \text{ alkenyl})S(CH_2)_n C_{3-10}$ heterocyclyl- R_w , $-(C_{2-6} \text{ alkenyl})-C_{3-10}$ heterocyclyl- R_w , $-(C_{2-6} \text{ alkenyl})-Z^1-C(=Z^2)N(R)_2$, $-(CH_2)_n SO_2R$, $-(CH_2)_n SO_3H$, $-(CH_2)_n PO(OR)_2$, C_{3-10} cycloalkyl, C_{6-10} aryl, C_{3-10} heterocyclyl, C_{2-6} alkenyl, and C_1-C_{10} alkyl, said alkyl, alkenyl, alkoxy, heterocyclyl and aryl optionally substituted with 1-3 groups selected from C_1-C_6 alkyl, halogen, $(CH_2)_n OH$, CN, NO_2 , $CON(R)_2$ and COOR;

20 Z^1 and Z^2 independently represents NR_w , O, CH_2 , or S;

m is 0-3;

n is 0-3 and

25 q is 0-2.

This and other aspects of the invention will be realized upon inspection of the invention as a whole.

30 DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel potassium channel blockers of Formula I. It also relates to a method for decreasing elevated intraocular pressure or treating glaucoma by administration, preferably topical or intra-cameral administration, of a composition containing a

potassium channel blocker of Formula I described hereinabove and a pharmaceutically acceptable carrier.

In another embodiment R_w is selected from H, C_{1-6} alkyl, $-C(O)C_{1-6}$ alkyl and $-C(O)N(R)_2$ and all other variables are as originally described..

Still another embodiment of this invention is realized when R_6 is C_{1-10} alkyl, $(CH_2)_n C_{3-8}$ cycloalkyl, said alkyl, optionally substituted with 1 to 3 groups of R^a , and all other variables are as originally described.

Yet another embodiment of this invention is realized when R_6 is C_{1-10} alkyl, optionally substituted with 1 to 3 groups of R^a , and all other variables are as originally described.

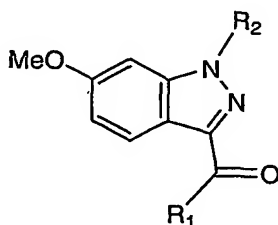
Yet another embodiment of this invention is realized when R_y is C_{1-6} alkyl, and all other variables are as originally described.

Still another embodiment of this invention is realized when R_2 is C_{1-10} alkyl or $(CH_2)_n C_{3-8}$ cycloalkyl and R_3 is C_{1-10} alkyl, or $(CH_2)_n C_{3-10}$ heterocyclyl, said heterocyclyl and alkyl optionally substituted with 1 to 3 groups of R^a . A subembodiment of this invention is realized when n is 0.

Another embodiment of the instant invention is realized when R^a is selected from F, Cl, Br, I, CF_3 , $N(R)_2$, NO_2 , CN, $-O-$, $-CONHR_8$, $-CON(R_8)_2$, $-O(CH_2)_n COOR$, $-NH(CH_2)_n OR$, $-COOR$, $-OCF_3$, $-NHCOR$, $-SO_2R$, $-SO_2NR_2$, $-SR$, $(C_1-C_6 \text{ alkyl})O-$, $-(CH_2)_n O(CH_2)_m OR$, $-(CH_2)_n C_{1-6}$ alkoxy, $(aryl)O-$, $-OH$, $(C_1-C_6 \text{ alkyl})S(O)_m-$, $H_2N-C(NH)-$, $(C_1-C_6 \text{ alkyl})C(O)-$, $(C_1-C_6 \text{ alkyl})OC(O)NH-$, $-(C_1-C_6 \text{ alkyl})NR_w(CH_2)_n C_{3-10}$ heterocyclyl- R_w , $-(CH_2)_n -Z^1-C(=Z^2)N(R)_2$, $-(C_{2-6} \text{ alkenyl})NR_w(CH_2)_n C_{3-10}$ heterocyclyl- R_w , $-(C_{2-6} \text{ alkenyl})-Z^1-C(=Z^2)N(R)_2$, $-(CH_2)_n SO_2R$, $-(CH_2)_n SO_3H$, $-(CH_2)_n PO(OR)_2$, C_{2-6} alkenyl, and C_1-C_{10} alkyl, said alkyl and alkenyl, optionally substituted with 1-3 groups selected from C_1-C_6 alkyl, and $COOR$;

Examples of compounds to be used in this invention are found in Table 1:

Table 1



R1	R2
<p> $x = \text{CH}, \text{N}$ $p = 0-1;$ $n = 0-3$ </p>	
$-\xi-(\text{CH}_2)_n\text{OH}$ $n=0-3$	

or a pharmaceutically acceptable salt, enantiomer, diastereomer or mixture thereof.

The invention is described herein in detail using the terms defined below unless otherwise specified.

The compounds of the present invention may have asymmetric centers, chiral axes and chiral planes, and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention. (See E.L. Eliel and S.H. Wilen *Stereochemistry of Carbon Compounds* (John Wiley and Sons, New York 1994), in particular pages 1119-1190)

When any variable (e.g. aryl, heterocycle, R¹, R⁶ etc.) occurs more than one time in any constituent, its definition on each occurrence is independent at every other occurrence. Also, combinations of substituents/or variables are permissible only if such combinations result in stable compounds.

When R^a is -O- and attached to a carbon it is referred to as a carbonyl group and when it is attached to a nitrogen (e.g., nitrogen atom on a pyridyl group) or sulfur atom it is referred to a N-oxide and sulfoxide group, respectively.

The term "alkyl" refers to a monovalent alkane (hydrocarbon) derived radical containing from 1 to 10 carbon atoms unless otherwise defined. It may be straight, branched or cyclic. Preferred alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, t-butyl, cyclopropyl cyclopentyl and cyclohexyl. When the alkyl group is said to be substituted with an alkyl group, this is used interchangeably with "branched alkyl group".

Cycloalkyl is a specie of alkyl containing from 3 to 15 carbon atoms, unless otherwise defined, without alternating or resonating double bonds between carbon atoms. It may contain from 1 to 4 rings, which are fused. Examples of such cycloalkyl elements include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

Alkenyl is C₂-C₆ alkenyl.

Alkoxy refers to an alkyl group of indicated number of carbon atoms attached through an oxygen bridge, with the alkyl group optionally substituted as described herein. Said groups are those groups of the designated length in either a straight or branched configuration and if two or more carbon atoms in length, they may include a double or a triple bond. Exemplary of such alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, tertiary butoxy, pentoxy, isopentoxy, hexoxy, isohexoxy allyloxy, propargyloxy, and the like.

Halogen (halo) refers to chlorine, fluorine, iodine or bromine.

Aryl refers to aromatic rings e.g., phenyl, substituted phenyl and the like, as well as rings which are fused, e.g., naphthyl, phenanthrenyl and the like. An aryl group thus

contains at least one ring having at least 6 atoms, with up to five such rings being present, containing up to 22 atoms therein, with alternating (resonating) double bonds between adjacent carbon atoms or suitable heteroatoms. Examples of aryl groups are phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthryl or acenaphthyl and phenanthrenyl, preferably phenyl, naphthyl or phenanthrenyl. Aryl groups may likewise be substituted as defined. Preferred substituted aryls include phenyl and naphthyl.

The term heterocyclyl or heterocyclic, as used herein, represents a stable 3- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. A fused heterocyclic ring system may include carbocyclic rings and need include only one heterocyclic ring. The term heterocycle or heterocyclic includes heteroaryl moieties.

Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, dihydropyrrolyl, 1,3-dioxolanyl, furyl, imidazolidinyl, imidazoliny, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-oxopiperazinyl, 2-oxopiperdinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, and thienyl. Preferably, heterocycle is selected from 2-azepinonyl, benzimidazolyl, 2-diazapinonyl, dihydroimidazolyl, dihydropyrrolyl, imidazolyl, 2-imidazolidinonyl, indolyl, isoquinolinyl, morpholinyl, piperidyl, piperazinyl, pyridyl, pyrrolidinyl, 2-piperidinonyl, 2-pyrimidinonyl, 2-pyrrolidinonyl, quinolinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, and thienyl.

The term "heteroatom" means O, S or N, selected on an independent basis.

The term "heteroaryl" refers to a monocyclic aromatic hydrocarbon group having 5 or 6 ring atoms, or a bicyclic aromatic group having 8 to 10 atoms, containing at least one heteroatom, O, S or N, in which a carbon or nitrogen atom is the point of attachment, and in which one or two additional carbon atoms is optionally replaced by a heteroatom selected from O or S, and in which from 1 to 3 additional carbon atoms are

optionally replaced by nitrogen heteroatoms, said heteroaryl group being optionally substituted as described herein. Examples of such heterocyclic elements include, but are not limited to, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnoliny, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl, imidazolyl, indoliny, indolyl, isochromanyl, isoindoliny, isoquinoliny, isothiazolyl, naphthyridiny, oxadiazolyl, pyridyl, pyraziny, pyrazolyl, pyridaziny, pyrimidiny, pyrrolyl, quinazoliny, quinoliny, quinoxaliny, tetrahydroisoquinoliny, tetrahydroquinoliny, thiazolyl, thienofuryl, thienothienyl, thienyl and triazolyl. Additional nitrogen atoms may be present together with the first nitrogen and oxygen or sulfur, giving, e.g., thiadiazole.

This invention is also concerned with compositions and methods of treating ocular hypertension or glaucoma by administering to a patient in need thereof one of the compounds of formula I in combination with a β -adrenergic blocking agent such as timolol, betaxolol, levobetaxolol, carteolol, levobunolol, a parasympathomimetic agent such as epinephrine, iopidine, brimonidine, clonidine, para-aminoclonidine, carbonic anhydrase inhibitor such as dorzolamide, acetazolamide, metazolamide or brinzolamide, an EP4 agonist (such as those disclosed in WO 02/24647, WO 02/42268, EP 1114816, WO 01/46140 and WO 01/72268), a prostaglandin such as latanoprost, travaprost, unoprostone, rescula, S1033 (compounds set forth in US Patent Nos. 5,889,052; 5,296,504; 5,422,368; and 5,151,444); a hypotensive lipid such as lumigan and the compounds set forth in US Patent No. 5,352,708; a neuroprotectant disclosed in US Patent No. 4,690,931, particularly eliprodil and R-eliprodil as set forth in WO 94/13275, including memantine; or an agonist of 5-HT₂ receptors as set forth in PCT/US00/31247, particularly 1-(2-aminopropyl)-3-methyl-1H-imidazol-6-ol fumarate and 2-(3-chloro-6-methoxy-indazol-1-yl)-1-methyl-ethylamine. An example of a hypotensive lipid (the carboxylic acid group on the α -chain link of the basic prostaglandin structure is replaced with electrochemically neutral substituents) is that in which the carboxylic acid group is replaced with a C₁₋₆ alkoxy group such as OCH₃ (PGF_{2a} 1-OCH₃), or a hydroxy group (PGF_{2a} 1-OH).

Preferred potassium channel blockers are calcium activated potassium channel blockers. More preferred potassium channel blockers are high conductance, calcium activated potassium (Maxi-K) channel blockers. Maxi-K channels are a family of ion channels that are prevalent in neuronal, smooth muscle and epithelial tissues and which are gated by membrane potential and intracellular Ca²⁺.

The present invention is based upon the finding that maxi-K channels, if blocked, inhibit aqueous humor production by inhibiting net solute and H₂O efflux and therefore lower

IOP. This finding suggests that maxi-K channel blockers are useful for treating other ophthalmological dysfunctions such as macular edema and macular degeneration. It is known that lowering IOP promotes blood flow to the retina and optic nerve. Accordingly, the compounds of this invention are useful for treating macular edema and/or macular degeneration.

5 It is believed that maxi-K channel blockers which lower IOP are useful for providing a neuroprotective effect. They are also believed to be effective for increasing retinal and optic nerve head blood velocity and increasing retinal and optic nerve oxygen by lowering IOP, which when coupled together benefits optic nerve health. As a result, this invention further relates to a method for increasing retinal and optic nerve head blood velocity, increasing retinal
10 and optic nerve oxygen tension as well as providing a neuroprotective effect or a combination thereof.

A number of marketed drugs function as potassium channel antagonists. The most important of these include the compounds Glyburide, Glipizide and Tolbutamide. These potassium channel antagonists are useful as antidiabetic agents. The compounds of this
15 invention may be combined with one or more of these compounds to treat diabetes.

Potassium channel antagonists are also utilized as Class 3 antiarrhythmic agents and to treat acute infarctions in humans. A number of naturally occurring toxins are known to block potassium channels including Apamin, Iberiotoxin, Charybdotoxin, Noxiustoxin, Kaliotoxin, Dendrotoxin(s), mast cell degranulating (MCD) peptide, and β -Bungarotoxin (β -
20 BTX). The compounds of this invention may be combined with one or more of these compounds to treat arrhythmias.

Depression is related to a decrease in neurotransmitter release. Current treatments of depression include blockers of neurotransmitter uptake, and inhibitors of enzymes involved in neurotransmitter degradation which act to prolong the lifetime of neurotransmitters.

25 Alzheimer's disease is also characterized by a diminished neurotransmitter release. Three classes of drugs are being investigated for the treatment of Alzheimer's disease cholinergic potentiators such as the anticholinesterase drugs (e.g., physostigmine (eserine), and Tacrine (tetrahydroaminocridine)); nootropics that affect neuron metabolism with little effect elsewhere (e.g., Piracetam, Oxiracetam; and those drugs that affect brain vasculature such as a mixture of
30 ergoloid mesylates and calcium channel blocking drugs including Nimodipine. Selegiline, a monoamine oxidase B inhibitor which increases brain dopamine and norepinephrine has reportedly caused mild improvement in some Alzheimer's patients. Aluminum chelating agents have been of interest to those who believe Alzheimer's disease is due to aluminum toxicity. Drugs that affect behavior, including neuroleptics, and anxiolytics have been employed.

Anxiolytics, which are mild tranquilizers, are less effective than neuroleptics. The present invention is related to novel compounds which are useful as potassium channel antagonists.

The compounds within the scope of the present invention exhibit potassium channel antagonist activity and thus are useful in disorders associated with potassium channel malfunction. A number of cognitive disorders such as Alzheimer's Disease, memory loss or depression may benefit from enhanced release of neurotransmitters such as serotonin, dopamine or acetylcholine and the like. Blockage of Maxi-K channels maintains cellular depolarization and therefore enhances secretion of these vital neurotransmitters.

The compounds of this invention may be combined with anticholinesterase drugs such as physostigmine (eserine) and Tacrine (tetrahydroaminocridine), nootropics such as Piracetam, Oxiracetam, ergoloid mesylates, selective calcium channel blockers such as Nimodipine, or monoamine oxidase B inhibitors such as Selegiline, in the treatment of Alzheimer's disease. The compounds of this invention may also be combined with Apamin, Iberiotoxin, Charybdotoxin, Noxiustoxin, Kaliotoxin, Dendrotoxin(s), mast cell degranulating (MCD) peptide, β -Bungarotoxin (β -BTX) or a combination thereof in treating arrhythmias. The compounds of this invention may further be combined with Glyburide, Glipizide, Tolbutamide or a combination thereof to treat diabetes.

The herein examples illustrate but do not limit the claimed invention. Each of the claimed compounds are potassium channel antagonists and are thus useful in the described neurological disorders in which it is desirable to maintain the cell in a depolarized state to achieve maximal neurotransmitter release. The compounds produced in the present invention are readily combined with suitable and known pharmaceutically acceptable excipients to produce compositions which may be administered to mammals, including humans, to achieve effective potassium channel blockage.

For use in medicine, the salts of the compounds of formula I will be pharmaceutically acceptable salts. Other salts may, however, be useful in the preparation of the compounds according to the invention or of their pharmaceutically acceptable salts. When the compound of the present invention is acidic, suitable "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as arginine, betaine

caffeine, choline, N,N¹-dibenzylethylenediamine, diethylamin, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine tripropylamine, tromethamine and the like.

When the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid and the like. Particularly preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric and tartaric acids.

The preparation of the pharmaceutically acceptable salts described above and other typical pharmaceutically acceptable salts is more fully described by Berg *et al.*, "Pharmaceutical Salts," *J. Pharm. Sci.*, 1977:66:1-19.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specific amounts, as well as any product which results, directly or indirectly, from combination of the specific ingredients in the specified amounts.

When a compound according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, sex and response of the individual patient, as well as the severity of the patient's symptoms.

The maxi-K channel blockers used can be administered in a therapeutically effective amount intravenously, subcutaneously, topically, transdermally, parenterally or any other method known to those skilled in the art. Ophthalmic pharmaceutical compositions are preferably adapted for topical administration to the eye in the form of solutions, suspensions, ointments, creams or as a solid insert. Ophthalmic formulations of this compound may contain from 0.01 ppm to 1% and especially 0.1 ppm to 1% of medicament. Higher dosages as, for example, about 10% or lower dosages can be employed provided the dose is effective in reducing intraocular pressure, treating glaucoma, increasing blood flow velocity or oxygen tension. For a single dose, from between 0.01 to 5000 ng, preferably 0.1 to 500 ng, and especially 1 to 100 ng of the compound can be applied to the human eye.

The pharmaceutical preparation which contains the compound may be conveniently admixed with a non-toxic pharmaceutical organic carrier, or with a non-toxic pharmaceutical inorganic carrier. Typical of pharmaceutically acceptable carriers are, for example, water, mixtures of water and water-miscible solvents such as lower alkanols or aralkanols, vegetable oils, polyalkylene glycols, petroleum based jelly, ethyl cellulose, ethyl oleate, carboxymethyl-cellulose, polyvinylpyrrolidone, isopropyl myristate and other conventionally employed acceptable carriers. The pharmaceutical preparation may also contain non-toxic auxiliary substances such as emulsifying, preserving, wetting agents, bodying agents and the like, as for example, polyethylene glycols 200, 300, 400 and 600, carbowaxes 1,000, 1,500, 4,000, 6,000 and 10,000, antibacterial components such as quaternary ammonium compounds, phenylmercuric salts known to have cold sterilizing properties and which are non-injurious in use, thimerosal, methyl and propyl paraben, benzyl alcohol, phenyl ethanol, buffering ingredients such as sodium borate, sodium acetates, gluconate buffers, and other conventional ingredients such as sorbitan monolaurate, triethanolamine, oleate, polyoxyethylene sorbitan monopalmitate, dioctyl sodium sulfosuccinate, monothioglycerol, thiosorbitol, ethylenediamine, tetracetic acid, and the like. Additionally, suitable ophthalmic vehicles can be used as carrier media for the present purpose including conventional phosphate buffer vehicle systems, isotonic boric acid vehicles, isotonic sodium chloride vehicles, isotonic sodium borate vehicles and the like. The pharmaceutical preparation may also be in the form of a microparticle formulation.

The pharmaceutical preparation may also be in the form of a solid insert. For example, one may use a solid water soluble polymer as the carrier for the medicament. The polymer used to form the insert may be any water soluble non-toxic polymer, for example, cellulose derivatives such as methylcellulose, sodium carboxymethyl cellulose, (hydroxyloweralkyl cellulose), hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose; acrylates such as polyacrylic acid salts, ethylacrylates, polyactylamides; natural products such as gelatin, alginates, pectins, tragacanth, karaya, chondrus, agar, acacia; the starch derivatives such as starch acetate, hydroxymethyl starch ethers, hydroxypropyl starch, as well as other synthetic derivatives such as polyvinyl alcohol, polyvinyl pyrrolidone, polyvinyl methyl ether, polyethylene oxide, neutralized carbopol and xanthan gum, gellan gum, and mixtures of said polymer.

Suitable subjects for the administration of the formulation of the present invention include primates, man and other animals, particularly man and domesticated animals such as cats and dogs.

The pharmaceutical preparation may contain non-toxic auxiliary substances such as antibacterial components which are non-injurious in use, for example, thimerosal, benzalkonium chloride, methyl and propyl paraben, benzylidodecinium bromide, benzyl alcohol,

or phenylethanol; buffering ingredients such as sodium chloride, sodium borate, sodium acetate, sodium citrate, or gluconate buffers; and other conventional ingredients such as sorbitan monolaurate, triethanolamine, polyoxyethylene sorbitan monopalmitate, ethylenediamine tetraacetic acid, and the like.

5 The ophthalmic solution or suspension may be administered as often as necessary to maintain an acceptable IOP level in the eye. It is contemplated that administration to the mamalian eye will be about once or twice daily.

 For topical ocular administration the novel formulations of this invention may take the form of solutions, gels, ointments, suspensions or solid inserts, formulated so that a unit
10 dosage comprises a therapeutically effective amount of the active component or some multiple thereof in the case of a combination therapy.

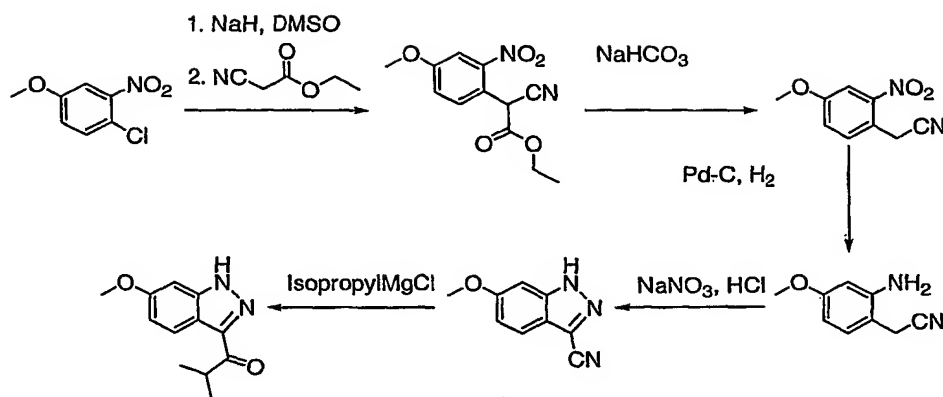
 The following examples given by way of illustration is demonstrative of the present invention.

Definitions of the terms used in the examples are as follows:

15 SM - Starting material,
 DMSO – dimethyl sulfoxide,
 TLC – thin layer chromatography,
 SGC – silica gel chromatography,
 PhMgBr – phenylmagnesiumbromide
20 h = hr = hour,
 THF – tetrahydrofuran,
 DMF – dimethylformamide,
 min – minute,
 LC/MS – liquid chromatography/mass spectrometry,
25 HPLC – high performance liquid chromatography,
 PyBOP – Benzotriazol-1-yloxytris-(dimethyl amino)phosphonium hexafluorophosphate,
 equiv = eq = equivalent,
 NBS – N-Bromosuccinamide and
 AIBN – 2,2'-azobisisobutyronitrile.

30 The compounds of this invention can be made, with modification where appropriate, in accordance with Scheme 1. Examples 1-3 are also produced in accordance with Scheme 1.

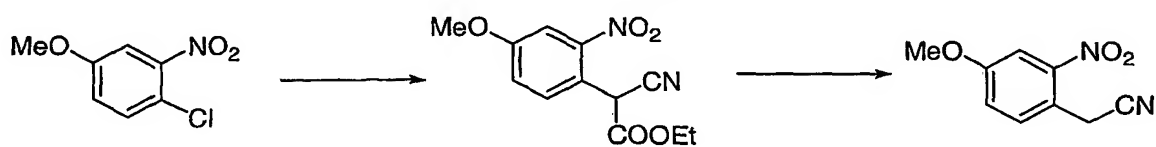
Scheme 1



In Scheme 1 nitroanisole is brominated using NBS, AIBN and benzoyl peroxide. Treatment of the bromonitroanisole with potassium cyanide yielded the cyanonitroanisole.

- 5 Conversion of the nitro group to an amine is accomplished by hydrogenation. The amine is then treated with sodium nitrite and HCl to yield the indazole ring. In this reaction as soon as the diazonium is generated by nitrosation of the aniline moiety it is trapped intramolecularly by the acidic benzyl cyanide. Tautomerization of the resultant derivative gives the indazole nucleus.
- 0 Treatment of the nitrile with a Grignard followed by hydrolysis of the resultant imino-magnesium complex gives the desired alkyl/aryl ketone.

Preparative Example 1

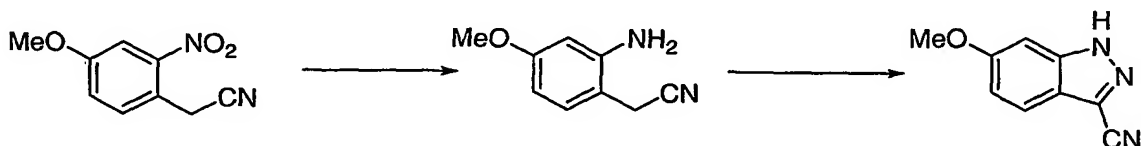


- 5 In a 500 mL flask was charged 336 mmoles (13.44g; 60%) of NaH. Under argon 150 mL of DMSO was added, followed by dropwise addition of 32 mL of ethyl cyanoacetate (2.2 equiv.; 352 mmloes) at 50 C. After all the addition the reaction was warmed upto room temperature over 1h. 30 g of starting nitro benzene derivative was added (160 mmoles) as a powder. The reaction mixture was heated in a closed system at 90oC for 8hours. Acidification and standard work-up gave a crude oily residue which was purified over a silica-gel column to
- 10 give 39 g of desired crystalline product which was decarboxylated to give the benzyl nitrile as follows. Thirty eight grams of SM obtained above was dissolved in 400 mL of 1N sodium carbonate. The homogenous solution was stirred at rt for two days. TLC analysis indicated

competition of reaction. The reaction mixture was acidified and extracted with ethyl acetate (100 mL X 4). The combined organic phases were dried over sodium sulphate and concentrated and residue was subjected to SGC to give the desired product.

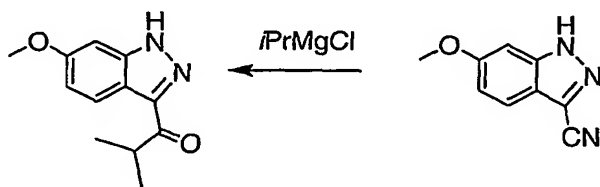
¹H NMR CDCl₃: 7.72 (1H, d, J = 3 Hz); 7.61 (1H, d, J = 8.5 Hz); 7.25 (1H, dd, J = 3 and 8.5 Hz); 4.17 (2H, s); 3.94 (3H, s). LCMS [M+H] = 193.

Preparative Example 2



10g of benzylnitrile derivative was dissolved in THF 20 mL followed by dilution with 50 mL of methanol. The reaction mixture was taken in a pressure tube, Pd-C (10% wt/ 10 mole %) was added and the reaction mixture was hydrogenated at 40 psi. After the requisite amount of hydrogen for the reduction of the NO₂ group was consumed the reaction was stopped. TLC analysis indicated a spot to spot conversion. The reaction mixture was filtered over a pad of celite and the filtrate was concentrated to a solid and used in the next step directly. Crude aniline derivative (52 mmol) was dissolved/suspended in 2N HCl (150 mL), cooled to 5 °C followed by the addition of 5.4g of sodium nitrite in 10 mL of water. The reaction mixture was allowed to stir for 1h with gradual warming to room temperature. TLC analysis indicated complete consumption of SM and the formation of a new spot. The reaction mixture was extracted with ethyl acetate (100 mL X 4); organic phase was collected, dried and concentrated. The residue was purified by SGC to give desired product. LCMS [M+H] = 174

Preparative Example 3



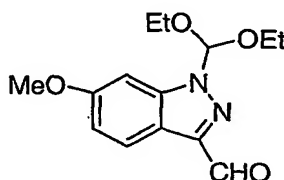
Weighed out 4.15 g of indazole and azeotroped water with 2 toluene (100 ml) washings, pulling off toluene azeotrope by rotovap. Dried thoroughly under high vacuum and performed argon purges. Dissolved in 40 ml dry THF and 92 ml dry ether under argon. Cooled to 5°C in ice water bath. Charged 3 eq of isopropylmagnesium chloride ((6 ml of a 2M solution in THF) and

stired for 0.5 hr at room temp. Carefully charged 1N HCl (240 ml) and stired for 1 h. Monitored reaction by TLC. Extracted with EtOAc, rotovaped and produced desired product.

LCMS [M+H] = 219

5

Preparative Example 4



Step A: 100 g of 2-fluoro-4-methoxy-acetophenone in 400 mL of ethylene glycol was stirred at room temperature with hydrazine (0.624 mol, 20 g) for 4h after which the reaction mixture was heated to 150 oC for 48h. TLC analysis indicated complete reaction. Partitioned the reaction mixture into dichloromethane and brine. Dried organic phase over sodium sulphate and evaporated to a solid. Re-crystallized from hexane/dicholomethane gave 6-methoxy-3-methyl-1H-indazole.

¹H NMR (CDCL₃): 7.5 (1H, d, 7.5 Hz); 6.8 (2H, m); 3.8 (3H, s); 2.55 (3H, s)

LCMS [M+H] = 163

78g of 6-methoxy-3-methyl-1H-indazole was dissolved in 1L of MeCN containing 1.1 equiv of tri-ethyl amine, 0.2 equiv of DMAP was cooled to -5 oC; followed by slow addition of Boc₂O (1.1 equiv) in 200 mL of MeCN. After 2h of stirring the reaction at rt the reaction mixture was evaporated to an oil which was partitioned between EtOAc and brine, dried over sodium sulphate and evaporated. The residue was applied to a short SGC and eluted with 15% EtOAc in hexane. Evaporation gave Boc-protected product.

¹H NMR (CDCL₃): 7.6 (1H, bs); 7.42 (1H, d, J = 7.5 Hz); 6.85 (1H, dd); 3.8 (3H, s); 2.5 (3H, s); 1.7 (9H, s)

LCMS [M+H] = 263

100 g of BOC-protected indazole was dissolved in 600 mL of CCl₄, followed by addition of 1.1 equiv of NBS and 0.2 equiv of Bz₂O. Reaction mix was vac-purged with argon and set to reflux for 5h in presence of light from a sun lamp. Reaction mixture was filtered over a pad of SG and concentrated. Residual oil was purified over a short SGC. Mono-bromide and mixed fractions of di-bromo derivative were obtained.

mono-bromide: ^1H NMR (CDCl_3): 7.7 (1H, d, 7.5 Hz); 7.6 (1H, bs); 6.95 (1H, dd); 4.7 (2H, s); 3.9 (3H, s); 1.7 (9H, s);

di-bromide: ^1H NMR (CDCl_3): 8.05 (1H, d, $J = 7.5$ Hz); 7.6 (1H, bs); 7.0 (1H, dd); 6.85 (1H, s); 3.9 (3H, s); 1.7 (9H, s);

To a solution of dibromide (23.2g) in acetic acid was added sodium acetate (22.5g). The mixture was placed in oil bath and refluxed for a couple of hours until reaction completed. The mixture was cooled to room temperature and then poured into ice/water to give desired compound as an off-white solid. The solid was isolated by filtration and dried over nitrogen atmosphere.

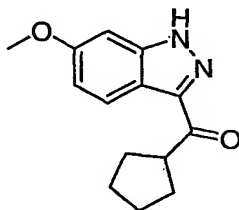
^1H NMR (CDCl_3) : δ 10.23 (1H, s); 8.19 (1H, d); 7.02 (1H, dd); 6.96 (1H, d); 3.90 (3H, s).

Step B:

To the intermediate from Step A was added triethyl orthoformate (40ml) and heated to 130°C for a couple of hours. The resulting mixture was concentrated to dry to give title compound as a brown solid.

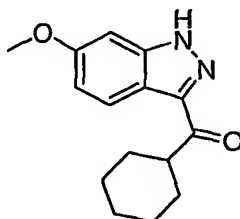
^1H NMR (DMSO) : δ 10.08 (1H, s); 7.98 (1H, d); 7.25 (1H, d); 7.02 (1H, dd); 6.81 (1H, s); 3.82 (3H, s); 3.52 (4H, q); 1.11 (6H, t).

Preparative Example 5



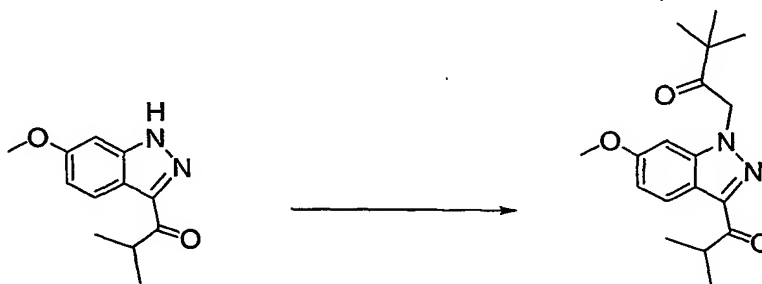
To a solution of intermediate from preparative Example 2 (1.00 g, 5.75 mmol) dissolved in THF (15 mL) was added cyclopentyl magnesium bromide (6.32 mL, 12.65 mmol) at 0°C . The reaction was allowed to warm to ambient temperature and was quenched with saturated NH_4Cl upon completion. The resulting reaction mixture was extracted with EtOAc and the combined organic layers were washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. The product was purified via SiO_2 gel chromatography to yield 580 mg of the desired product. ^1H NMR (CDCl_3) δ : 1.702 (2 H, m), 1.803 (2 H, m), 2.005 (4 H, m), 3.904 (3 H, s), 4.070 (1 H, m), 6.915 (1 H, s), 7.010 (1 H, d), 8.272 (1 H, d).

Preparative Example 6



The desired compound was prepared by a procedure similar to the one described for Preparative Example 5, but cyclohexyl magnesium bromide was used in place of cyclopentyl magnesium bromide. ^1H NMR (CDCl_3) δ : 1.327 (1 H, m), 1.479 (2 H, m), 1.604 (2 H, m), 1.781 (1 H, m), 1.861 (2 H, m), 2.000 (2 H, m), 3.641 (1 H, m), 3.902 (3 H, s), 6.923 (1 H, s), 7.008 (1 H, d), 8.259 (1 H, d).

Example 1

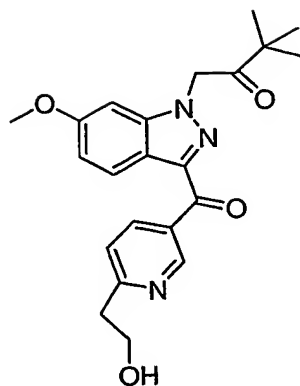


Indazole (0.60 mmoles from Preparative Example 3) starting material obtained as above was dissolved in DMF (3 mL) followed by the addition of sodium hydride (0.88 mmoles). The reaction was stirred at room temperature for 15 min, followed by the addition of tert-butyl bromoacetate (0.669 mmoles). The reaction was stirred at room temperature for 30 min. TLC and LC-MS analysis indicated complete consumption of starting material concurrent with the formation of a new product spot. The reaction mixture was quenched by the addition of water. Standard aqueous work-up followed by purification of crude by SGC gave the desired product as white solid.

^1H NMR in CDCl_3 : 8.22 (1H, d, $J = 9$ Hz); 6.97 (1H, dd, $J = 2$ and 9 Hz); 6.5 (1H, d, $J = 2$ Hz); 5.4 (2H, s); 3.94 (3H, s); 2.8 (1H, m); 1.38 (9H, s); 1.27 (6H, d, $J = 6.5$ Hz).

LCMS = $[\text{M}+\text{H}] = 317$

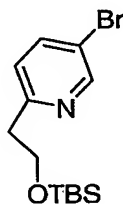
Example 2



1-(3-([6-(2 hydroxyethyl)pyridine-3-yl]carbonyl)-6-methoxy-1H-indazol-1-yl)-3,3-dimethylbutan-2-one

5

Step A:



To a solution of 2,5-dibromopyridine (2.4g) in toluene was added tributylallyltin (3.4 ml) and dichlorobis(triphenylphosphine) palladium (0.7g) under nitrogen atmosphere. The mixture was refluxed for a couple of hours and concentrated under reduced pressure. The residue was re-dissolved in "wet ether" and added DBU (3ml) slowly to give a cloudy solution. The mixture was filtered over a pad of silica gel and concentrated. The residue was dissolved in methylene chloride/methanol=1/1 solution and cooled to -78°C . To this solution was bubbled though ozone until the reaction mixture became a blue color. The reaction was warmed to 0°C and added sodium borohydride (0.5g) portion-wise. After stirring at 0°C for 1 hour, the mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with 1N NaOH_{aq} , brine, dried (MgSO_4), and concentrated under reduced pressure to afford crude alcohol. The alcohol was purified by silica gel (methylene chloride/ ethyl acetate=1/1) to give desired alcohol. To a solution of alcohol in methylene chloride was added imidazole (0.4g) and TBS-Cl (0.8g) at 0°C . The mixture was stirred for 1 hour. The reaction was poured into 0.1 N HCl_{aq} extracted with methylene chloride. The organic layer was washed with brine, dried (MgSO_4) and

10

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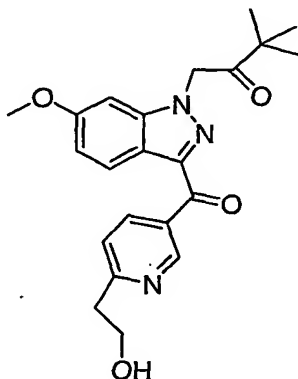
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evaporated. The residue was purified by silica gel (100% methylene chloride) to give desired compound.

^1H NMR (CDCl_3) : δ 8.61 (1H, d); 7.73 (1H, dd); 7.14 (1H, d); 3.97 (2H, t); 2.96 (2H, t); 0.86 (9H, s); -0.02 (6H, s).

5

Step B:



The desired compound was prepared by a procedure similar to the one described for Example 5, Steps 5, 6, 7, and 8. This compound was purified by silica gel (hexanes/ ethyl acetate=1/3).

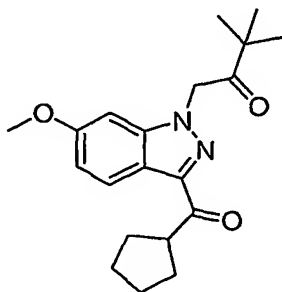
10 ^1H NMR (CHCl_3) : δ 9.53 (1H, d); 8.54 (1H, dd); 8.35 (1H, d); 7.37 (1H, d); 7.07 (1H, dd); 6.56 (1H, d); 5.45 (2H, s); 4.11 (2H, t); 3.90 (3H, s); 3.18 (2H, t); 1.38 (9H, s).

LC-MS ($\text{M}+\text{H}$)=396.2.

15 Examples 3-5 as shown below are made, with some modification of the desired compound of Preparative Example 6, by alkylation of the indazole as described in Example 1. Additionally, analogs of Examples 1 and 4 can be prepared following analogous procedures using the indazole of Preparative Example 4 or alternatively another indazole prepared following procedures described herein.

20

Example 3

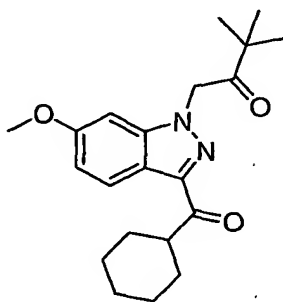


To 195 mg of NaH (60 % dispersion in oil washed with hexane) was added DMF (10 mL) and Intermediate 3 (597 mg, 2.44 mmol). The reaction stirred at room temperature for 30 min before 1-chloropinacolone (3.81 mL, 2.92 mmol) was added. After 20 min the reaction was quenched with H₂O and diluted with EtOAc. The aqueous layer was extracted with EtOAc and the
5 combined organic layers were washed with H₂O, brine, dried over MgSO₄, and concentrated *in vacuo*. The crude material was purified via silica gel chromatography to yield.

¹H NMR (CDCl₃) δ : 1.361 (9 H, s), 1.683 (2 H, m), 1.788 (2 H, m), 1.974 (4 H, m), 3.872 (3 H, s), 4.029 (1 H, m), 5.372 (2 H, s), 6.514 (1 H, s), 6.986 (1 H, d), 8.267 (1 H, d).

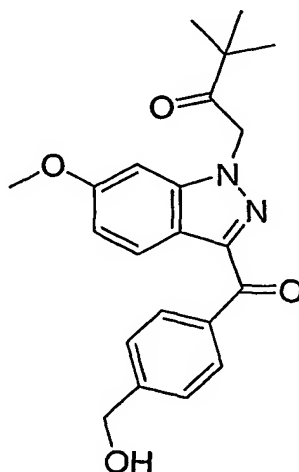
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Example 4



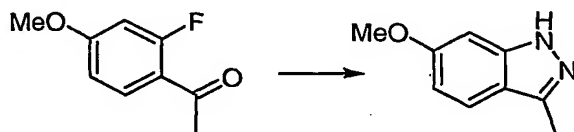
Using Preparative Example 8, this compound was prepared as described in Example 3. The title compound was purified via SiO₂ preparatory plate chromatography. ¹H NMR (CDCl₃) δ : 1.285-1.575 (15 H, m), 1.833 (2 H, d), 1.994 (2 H, d), 3.615 (1 H, m), 3.860 (3 H, s), 5.372 (2 H, s),
15 6.490 (1 H, s), 6.981 (1 H, d), 8.254 (1 H, d).

Example 5



di-tert--butyl 4-{{[1-(3,3-dimethyl-2-oxobutyl)-6-methoxy-1-H-indazole-3-yl]carbonyl}hydroxyl ethyl benzyl

Step 1

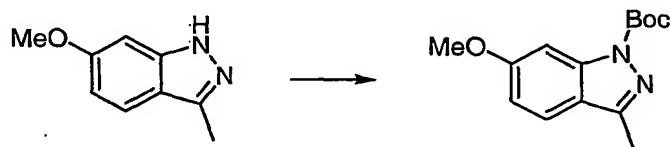


100 g of fluoro-acetophenone in 400 mL of ethylene glycol was stirred at room temperature with hydrazine (0.624 mol, 20 g) for 4h after which the reaction mixture was heated to 150 C for 48h. TLC analysis indicated complete reaction. Partitioned the reaction mixture into dichloromethane and brine. Dried organic phase over sodium sulphate and evaporated to a solid. Re-crystallized from hexane/dichloromethane gave indazole.

¹H NMR (CDCl₃): 7.5 (1H, d, 7.5 Hz); 6.8 (2H, m); 3.8 (3H, s); 2.55 (3H, s)

LCMS [M+H] = 163

Step 2



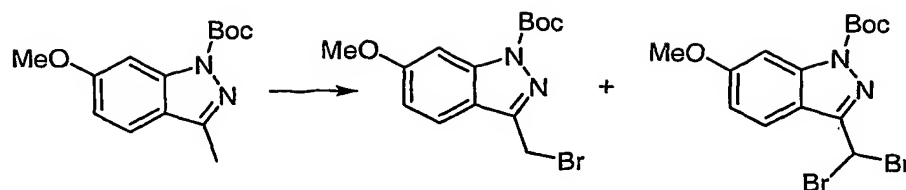
6-methoxy-3-methyl-1-H
indazole

tert-butyl-6-methoxy-3-methyl-
1-indazole-carboxylate

78g of indazole was dissolved in 1L of MeCN containing 1.1 equiv of tri-ethyl amine, 0.2 equiv of DMAP was cooled to -5 C; followed by slow addition of Boc₂O (1.1 equiv) in 200 mL of MeCN. After 2h of stirring the reaction at rt the reaction mixture was evaporated to an oil which was partitioned between EtOAc and brine, dried over sodium sulphate and evaporated. The residue was applied to a short SGC and eluted with 15% EtOAc in hexane. Evaporation gave product.

¹H NMR (CDCl₃): 7.6 (1H, bs); 7.42 (1H, d, J = 7.5 Hz); 6.85 (1H, dd); 3.8 (3H, s); 2.5 (3H, s); 1.7 (9H, s)

LCMS [M+H] = 263

Step 3

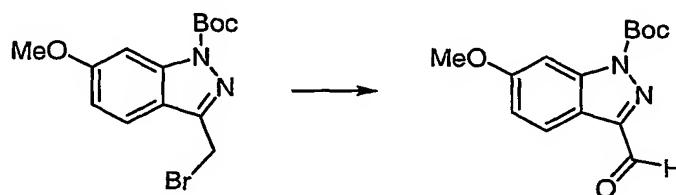
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100 g of indazole was dissolved in 600 mL of CCl₄, followed by addition of 1.1 equiv of NBS and 0.2 equiv of Bz₂O. Reaction mix was vac-purged with argon and set to reflux for 5h in presence of light from a sun lamp. Reaction mixture was filtered over a pad of SG and concentrated. Residual oil was purified over a short SGC. 85 g of pure bromide was obtained.

10 Mixed fractions yielded di-bromo derivative

mono-bromide: ¹H NMR (CDCl₃): 7.7 (1H, d, 7.5 Hz); 7.6 (1H, bs); 6.95 (1H, dd); 4.7 (2H, s); 3.9 (3H, s); 1.7 (9H, s);

15 di-bromide: ¹H NMR (CDCl₃): 8.05 (1H, d, J = 7.5 Hz); 7.6 (1H, bs); 7.0 (1H, dd); 6.85 (1H, s); 3.9 (3H, s); 1.7 (9H, s);

Step 4

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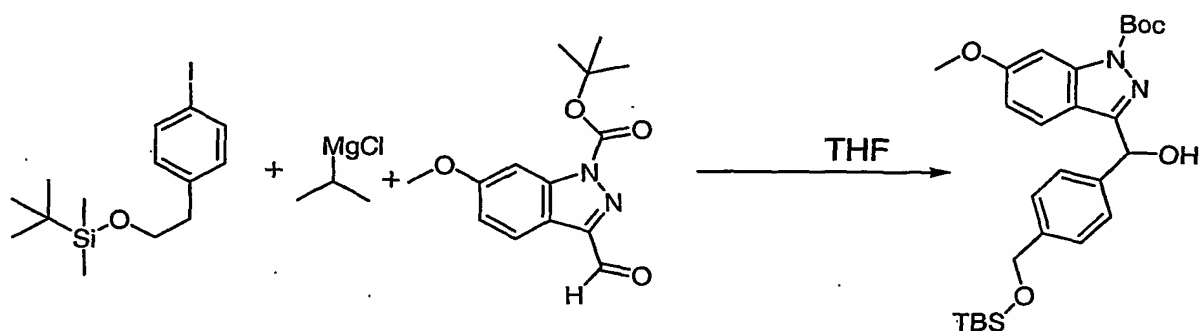
3-(bromomethyl)-6-methoxy-
1-H-indazole

t-butyl-3-formyl-6-methoxy-
1-H-indazole-1-carboxylate

5 g of bromide was dissolved in 10 mL of DMSO, cooled to 0 C followed by addition of 2.5 equiv of TMANO (trimethyl amine N-oxide). Reaction was stirred for 0.5h then a standard work-up and SG pad filtration gave desired product quantitatively. LCMS $[M+H] = 277$

5 1H NMR ($CDCl_3$): 10.2 (1H, s); 8.1 (1H, d, $J = 7.5$ Hz); 7.6 (1H, bs); 7.0 (1H, dd); 3.9 (3H, s); 1.7 (9H, s);

Step 5



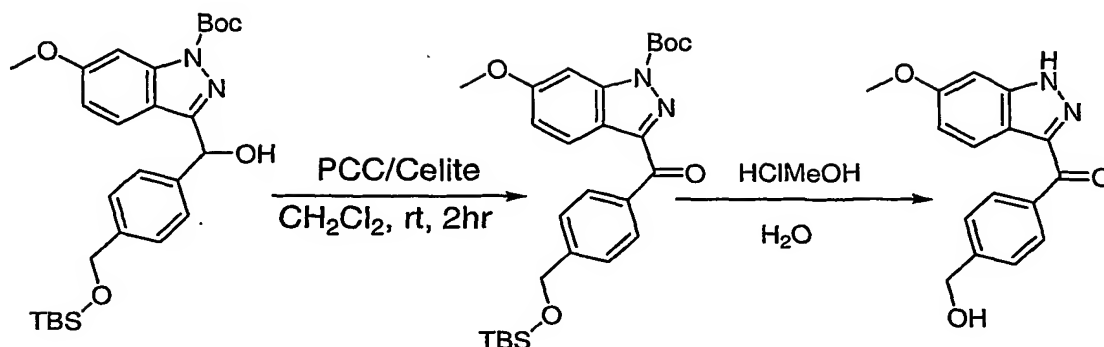
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Glasswares were flame dried under high vacuum

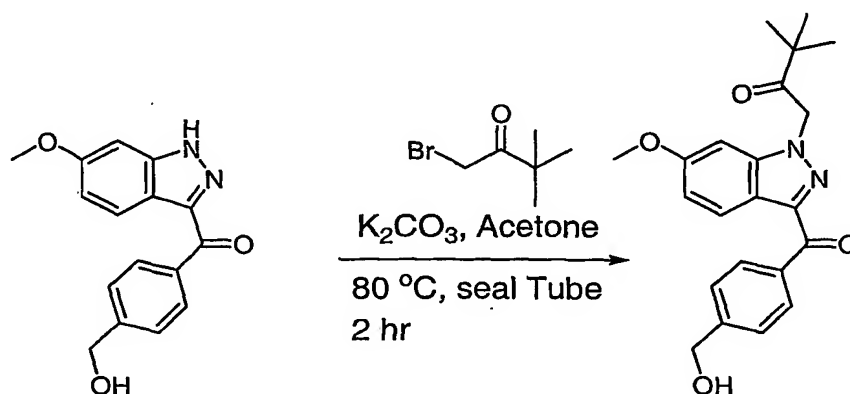
To neat iodo-benzyl alcohol derivative (3.6 g, 10 mmol) in the flask was slowly added isopropylMgCl (5 mL, 2M solution). After stirring at rt for 2 hr, indazole derivative (1.1g, 4 mmol) in 15 mL THF was added. The reaction mixture was stirred at rt for 2 hr. LC-MS

15 showed the reaction was complete. Pour the reaction mixture into 30 mL saturated NH_4Cl , followed by adding 40 mL ether. The organic layer was separated, the aqueous layer was extracted by ether (40 mL). The combined organic layers were washed with saturated K_2CO_3 (2x30 mL), water (40 mL) and brine (20 mL). The solvent was removed, the residue was used for next step reaction without further purification. LCMS $[M+H] = 499$

20

Steps 6 and 7

To a solution of indazole (crude from step 5) in 20 mL dichloromethane was added 5 g celite and 4.3 g of PCC (MW 215.56, ~2 eq). The reaction mixture was stirred at rt for 2 hr. LC-MS showed the reaction was completed LCMS [M+H] = 497. The reaction mixture was filtered. The solvent was removed, the residue was dissolved in 10 mL MeOH, and added 20 mL 2N HCl. After stirring for 1 hr at rt LCMS and TLC analysis indicated complete reaction. The reaction mixture was extracted with EtOAc (2x30 mL). The solvent was removed, the residue was used for next step reaction without further purification. LCMS [M+H] = 283

Step 8

To a solution of indazole (342 mg crude prod from step 7, ~10 mmol) in 15 mL acetone was added 1.5 g of K₂CO₃ and 1.5 mL Bromopinacolone (Mw 179.06, d 1.326, 2.0 g, 11 mmol). The reaction mixture was stirred at 80 °C in a seal tube for 2 hr. After filtered off salts, the solvent was removed, the residue was purified by HPFC to give white solid product.

^1H NMR (CDCl_3) = 8.3 (3H, m); 7.5 (1H, d, $J = 7.5$ Hz); 7.05 (1H, dd); 7.6 (1H, bs); 5.4 (2H, s); 4.8 (2H, bs); 3.9 (3H, s); 1.38 (9H, s)
LCMS $[\text{M}+\text{H}] = 381$

5 FUNCTIONAL ASSAYS

A. Maxi-K Channel

The identification of inhibitors of the Maxi-K channel can be accomplished using Aurora Biosciences technology, and is based on the ability of expressed Maxi-K channels to set cellular resting potential after transient transfection of both α and β subunits of the channel in
0 TsA-201 cells. In the absence of inhibitors, cells display a hyperpolarized membrane potential, negative inside, close to E_K (-80 mV) which is a consequence of the activity of the Maxi-K channel. Blockade of the Maxi-K channel will cause cell depolarization. Changes in membrane potential can be determined with voltage-sensitive fluorescence resonance energy transfer (FRET) dye pairs that use two components, a donor coumarin (CC_2DMPE) and an acceptor
5 oxanol ($\text{DiSBAC}_2(3)$). Oxanol is a lipophilic anion and distributes across the membrane according to membrane potential. Under normal conditions, when the inside of the cell is negative with respect to the outside, oxanol is accumulated at the outer leaflet of the membrane and excitation of coumarin will cause FRET to occur. Conditions that lead to membrane depolarization will cause the oxanol to redistribute to the inside of the cell, and, as a
10 consequence, to a decrease in FRET. Thus, the ratio change (donor/acceptor) increases after membrane depolarization.

Transient transfection of the Maxi-K channel in TsA-201 cells can be carried out as previously described (Hanner et al. (1998) J. Biol. Chem. 273, 16289-16296) using FUGENE6TM as the transfection reagent. Twenty four hours after transfection, cells are collected
15 in Ca^{2+} - Mg^{2+} -free Dulbecco's phosphate-buffered saline (D-PBS), subjected to centrifugation, plated onto 96-well poly-d-lysine coated plates at a density of 60,000 cells/well, and incubated overnight. The cells are then washed 1x with D-PBS, and loaded with 100 μl of 4 μM CC_2DMPE -0.02% pluronic-127 in D-PBS. Cells are incubated at room temperature for 30 min in the dark. Afterwards, cells are washed 2x with D-PBS and loaded with 100 μl of 6 μM
20 $\text{DiSBAC}_2(3)$ in (mM): 140 NaCl, 0.1 KCl, 2 CaCl_2 , 1 MgCl_2 , 20 Hepes-NaOH, pH 7.4, 10 glucose. Test compounds are diluted into this solution, and added at the same time. Cells are incubated at room temperature for 30 min in the dark.

Plates are loaded into a voltage/ion probe reader (VIPR) instrument, and the fluorescence emission of both CC_2DMPE and $\text{DiSBAC}_2(3)$ are recorded for 10 sec. At this
25 point, 100 μl of high-potassium solution (mM): 140 KCl, 2 CaCl_2 , 1 MgCl_2 , 20 Hepes-KOH, pH

7.4, 10 glucose are added and the fluorescence emission of both dyes recorded for an additional 10 sec. The ratio $CC_2DMPE/DiSBAC_2(3)$, before addition of high-potassium solution equals 1. In the absence of any inhibitor, the ratio after addition of high-potassium solution varies between 1.65-2.0. When the Maxi-K channel has been completely inhibited by either a known standard or test compound, this ratio remains at 1. It is possible, therefore, to titrate the activity of a Maxi-K channel inhibitor by monitoring the concentration-dependent change in the fluorescence ratio.

The compounds of this invention were found to cause concentration-dependent inhibition of the fluorescence ratio with IC_{50} 's in the range of about 1 nM to about 20 μ M, more preferably from about 10 nM to about 500 nM.

B. Electrophysiological assays of compound effects on high-conductance calcium-activated potassium channels

Human non-pigmented ciliary epithelial cells

The activity of high-conductance calcium-activated potassium (maxi-K) channels in human non-pigmented ciliary epithelial cells was determined using electrophysiological methods. Currents through maxi-K channels were recorded in the inside-out configuration of the patch clamp technique, where the pipette solution faces the extracellular side of the channel and the bath solution faces the intracellular side. Excised patches contained one to about fifty maxi-K channels. Maxi-K channels were identified by their large single channel conductance (250-300 pS), and by sensitivity of channel gating to membrane potential and intracellular calcium concentration. Membrane currents were recorded using standard electrophysiological techniques. Glass pipettes (Garner 7052) were pulled in two stages with a Kopf puller (model 750), and electrode resistance was 1-3 megohms when filled with saline. Membrane currents were recorded with EPC9 (HEKA Instruments) or Axopatch 1D (Axon Instruments) amplifiers, and digital conversion was done with ITC-16 interfaces (Instrutech Corp). Pipettes were filled with (mM); 150 KCl, 10 Hepes, 1 $MgCl_2$, 0.01 $CaCl_2$, 3.65 KOH, pH 7.20. The bath (intracellular) solution was identical, except, in some cases, calcium was removed, 1 mM EGTA was added and 20 mM KCl was replaced with 20 mM KF to eliminate calcium to test for calcium sensitivity of channel gating. Drugs were applied to the intracellular side of the channel by bath perfusion.

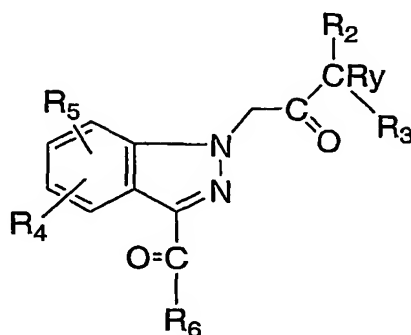
Human non-pigmented ciliary epithelial cells were grown in tissue culture as described (Martin-Vasallo, P., Ghosh, S., and Coca-Prados, M., 1989, J. Cell. Physiol. 141, 243-252), and plated onto glass cover slips prior to use. High resistance seals (>1 Gohm) were

formed between the pipette and cell surface, and inside out patches were excised. Maxi-K channels in the patch were identified by their gating properties; channel open probability increased in response to membrane depolarization and elevated intracellular calcium. In patches used for pharmacological analysis, removing intracellular calcium eliminated voltage-gated currents. Maxi-K currents were measured after depolarizing voltage steps or ramps that caused channel opening.

The compounds of this invention were applied to the intracellular side of the channel in appropriate concentrations (0.001 to 100 μ M). The compounds reduced channel open probability, and this effect was reversed upon washout of compounds from the experimental chamber. The IC₅₀ for block of maxi-K channels under these conditions for the compounds of this invention ranged from about 0.5 nM to about 10 μ M.

WHAT IS CLAIMED IS:

1. A compound of the structural formula I:



Formula I

or a pharmaceutically acceptable salt, enantiomer, diastereomer or mixture thereof:

wherein,

R represents hydrogen, or C₁₋₆ alkyl;

10 RY represents H, or C₁₋₆ alkyl;

R_w represents H, C₁₋₆ alkyl, -C(O)C₁₋₆ alkyl, -C(O)OC₁₋₆ alkyl, -SO₂N(R)₂, -SO₂C₁₋₆ alkyl, -SO₂C₆₋₁₀ aryl, NO₂, CN or -C(O)N(R)₂;

R₂ represents hydrogen, C₁₋₁₀ alkyl, OH, C₂₋₆ alkenyl, -(CH₂)_nO(CH₂)_mOR, -(CH₂)_nC₁₋₆ alkoxy, -(CH₂)_nC₃₋₈ cycloalkyl, -(CH₂)_nC₃₋₁₀ heterocyclyl, or -(CH₂)_nC₆₋₁₀ aryl, said alkyl,

15 heterocyclyl, or aryl optionally substituted with 1-3 groups selected from R^a;

R₃ represents hydrogen, C₁₋₁₀ alkyl, -(CH₂)_nC₃₋₈ cycloalkyl, -(CH₂)_nC₃₋₁₀ heterocyclyl, -(CH₂)_nCOOR, -(CH₂)_nC₆₋₁₀ aryl, nitro, cyano or halogen, said alkyl, heterocyclyl, or aryl optionally substituted with 1-3 groups of R^a;

20 R₄ and R₅ independently represent hydrogen, C₁₋₆ alkoxy, OH, C₁₋₆ alkyl, COOR, SO_qC₁₋₆ alkyl, COC₁₋₆ alkyl, SO₃H, -O(CH₂)_nN(R)₂, -O(CH₂)_nCO₂R, -OPO(OH)₂, CF₃, OCF₃ - N(R)₂, nitro, cyano, C₁₋₆ alkylamino, or halogen; and

25 R₆ represents hydrogen, C₁₋₁₀ alkyl, -(CH₂)_nC₆₋₁₀ aryl, -(CH₂)_nC₃₋₁₀ heterocyclyl, -(CH₂)_nC₃₋₈ cycloalkyl, said aryl, heterocyclyl and alkyl optionally substituted with 1-3 groups selected from R^a, wherein the R^a(s) can be attached to any carbon atom or heteroatom selected from N and S;

R₈ represents -(CH₂)_nC₃₋₈ cycloalkyl, -(CH₂)_n 3-10 heterocyclyl, C₁₋₆ alkoxy or -(CH₂)_nC₅₋₁₀ heteroaryl, -(CH₂)_nC₆₋₁₀ aryl said heterocyclyl, aryl or heteroaryl optionally substituted with 1-3 groups selected from R^a;

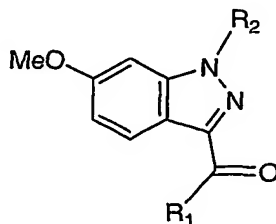
- 5 R^a represents F, Cl, Br, I, CF₃, N(R)₂, NO₂, CN, -O-, -COR₈, -CONHR₈, -CON(R₈)₂, -O(CH₂)_nCOOR, -NH(CH₂)_nOR, -COOR, -OCF₃, CF₂CH₂OR, -NHCOR, -SO₂R, -SO₂NR₂, -SR, (C₁-C₆ alkyl)O-, -(CH₂)_nO(CH₂)_mOR, -(CH₂)_nC₁₋₆ alkoxy, (aryl)O-, -(CH₂)_nOH, (C₁-C₆ alkyl)S(O)_m-, H₂N-C(NH)-, (C₁-C₆ alkyl)C(O)-, (C₁-C₆ alkyl)OC(O)NH-, -(C₁-C₆ alkyl)NR_w(CH₂)_nC₃₋₁₀ heterocyclyl-R_w, -(C₁-C₆ alkyl)O(CH₂)_nC₃₋₁₀ heterocyclyl-R_w, -(C₁-C₆ alkyl)S(CH₂)_nC₃₋₁₀ heterocyclyl-R_w, -(C₁-C₆ alkyl)-C₃₋₁₀ heterocyclyl-R_w, -(CH₂)_n-Z¹-C(=Z²)N(R)₂, -(C₂₋₆ alkenyl)NR_w(CH₂)_nC₃₋₁₀ heterocyclyl-R_w, -(C₂₋₆ alkenyl)O(CH₂)_nC₃₋₁₀ heterocyclyl-R_w, -(C₂₋₆ alkenyl)S(CH₂)_nC₃₋₁₀ heterocyclyl-R_w, -(C₂₋₆ alkenyl)-C₃₋₁₀ heterocyclyl-R_w, -(C₂₋₆ alkenyl)-Z¹-C(=Z²)N(R)₂, -(CH₂)_nSO₂R, -(CH₂)_nSO₃H, -(CH₂)_nPO(OR)₂, C₃₋₁₀cycloalkyl, C₆₋₁₀ aryl, C₃₋₁₀ heterocyclyl, C₂₋₆ alkenyl, and C₁-C₁₀ alkyl, said alkyl, alkenyl, alkoxy, heterocyclyl and aryl optionally substituted with 1-3 groups selected from C₁-C₆ alkyl, halogen, (CH₂)_nOH, CN, NO₂, CON(R)₂ and COOR;
- 15 Z¹ and Z² independently represents NR_w, O, CH₂, or S;
m is 0-3;
n is 0-3 and
20 q is 0-2.


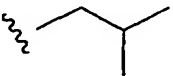

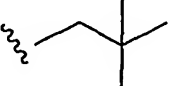

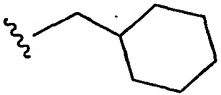



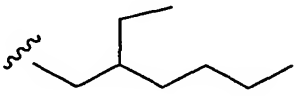

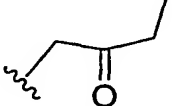

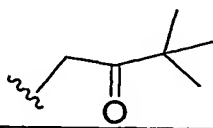
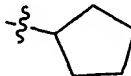
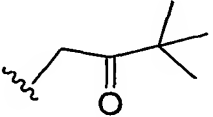
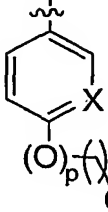
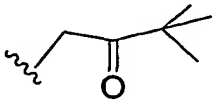
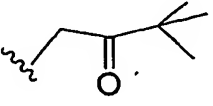
2. The compound according to claim 1 wherein R₆ is C₁₋₁₀ alkyl, or (CH₂)_nC₃₋₈ cycloalkyl and R_y is C₁₋₆ alkyl, said alkyl, optionally substituted with 1 to 3 groups of R^a.

3. The compound according to claim 1 wherein R₂ is C₁₋₁₀ alkyl or -(CH₂)_nC₃₋₈ cycloalkyl and R₃ is C₁₋₁₀ alkyl, or (CH₂)_nC₃₋₁₀ heterocyclyl, said heterocyclyl and alkyl optionally substituted with 1 to 3 groups of R^a.

4. A compound which is:

Table 1



R1	R2
	
	
	
	
	
	
	
	
 <p> $x = \text{CH}, \text{N}$ $p = 0-1;$ $n = 0-3$ </p>	
$-\overset{\xi}{\underset{\text{OH}}{\text{C}}}-(\text{CH}_2)_n\text{OH}$ $n = 0-3$	

or a pharmaceutically acceptable salt, enantiomer, diastereomer or mixture thereof.

5 5. A method for treating ocular hypertension or glaucoma comprising administration to a patient in need of such treatment a therapeutically effective amount of a compound of structural formula I of claim 1.

10 6. A method for treating macular edema, macular degeneration, increasing retinal and optic nerve head blood velocity, increasing retinal and optic nerve oxygen tension, and/or a neuroprotective effect comprising administration to a patient in need of such treatment a pharmaceutically effective amount of a compound of claim 1; or a pharmaceutically acceptable salt, enantiomer, diastereomer or mixture thereof.

15 7. A method of preventing repolarization or hyperpolarization of a mammalian cell containing potassium channel or a method of treating Alzheimer's Disease, depression, cognitive disorders, and/or arrhythmia disorders in a patient in need thereof comprising administering a pharmaceutically effective amount of a compound according to Claim 1, or a pharmaceutically acceptable salt, enantiomer, diastereomer or mixture thereof.

20 8. A method of treating diabetes in a patient in need thereof comprising administering a pharmaceutically effective amount of a compound according to claim 1, or a pharmaceutically acceptable salt, enantiomer, diastereomer or mixture thereof.

25 9. A composition comprising a compound of formula I of claim 1 and a pharmaceutically acceptable carrier.

30 10. The composition according to Claim 9 wherein the compound of formula I is applied as a topical formulation, said topical formulation administered as a solution or suspension and optionally containing xanthan gum or gellan gum.

35 11. A composition according to claim 9 wherein an active ingredient belonging to the group consisting of: β -adrenergic blocking agent, parasympatho-mimetic agent, sympathomimetic agent, carbonic anhydrase inhibitor, EP4 agonist, a prostaglandin or derivative thereof, hypotensive lipid, neuroprotectant, and/or 5-HT₂ receptor agonist is optionally added.

12. A composition according to claim 11 wherein the β -adrenergic blocking agent is timolol, betaxolol, levobetaxolol, carteolol, or levobunolol; the parasympathomimetic agent is pilocarpine; the sympathomimetic agent is epinephrine, brimonidine, iopidine, clonidine, or para-aminoclonidine, the carbonic anhydrase inhibitor is dorzolamide, acetazolamide, metazolamide or brinzolamide; the prostaglandin is latanoprost, travaprostone, unoprostone, rescula, or S1033, the hypotensive lipid is lumigan, the neuroprotectant is eliprotil, R-eliprotil or memantine; and the 5-HT₂ receptor agonist is 1-(2-aminopropyl)-3-methyl-1H-imidazol-6-ol fumarate or 2-(3-chloro-6-methoxy-indazol-1-yl)-1-methyl-ethylamine.
- 5

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 03/35080

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07D231/56 A61K31/416 A61P27/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01/52876 A (KACZOROWSKI GREGORY J ; GARCIA MARIA L (US); MCMANUS OWEN B (US); MERC) 26 July 2001 (2001-07-26) page 2, line 30 - page 3, line 25	1-12
A	WO 01/70701 A (ALCON UNIVERSAL LTD ; MAY JESSE A (US); FENG ZIXIA (US)) 27 September 2001 (2001-09-27) page 3, line 9 - page 4, line 30	1-12
A	WO 01/70702 A (ALCON UNIVERSAL LTD ; DANTANARAYANA ANURA P (US); MAY JESSE A (US); FE) 27 September 2001 (2001-09-27) page 3, line 1 - page 4, line 33	1-12

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

15 March 2004

Date of mailing of the international search report

23/03/2004

Name and mailing address of the ISA

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Authorized officer

Usueli, A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/35080

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 5-8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 03/35080

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0152876	A	26-07-2001	AU 3096301 A	31-07-2001
			CA 2396319 A1	26-07-2001
			EP 1251862 A1	30-10-2002
			JP 2003520245 T	02-07-2003
			WO 0152876 A1	26-07-2001
			US 2003153613 A1	14-08-2003
			US 2001044460 A1	22-11-2001
WO 0170701	A	27-09-2001	AU 1918001 A	03-10-2001
			BR 0017163 A	14-01-2003
			CA 2401959 A1	27-09-2001
			CN 1450995 T	22-10-2003
			EP 1268439 A1	02-01-2003
			JP 2003535821 T	02-12-2003
			WO 0170701 A1	27-09-2001
WO 0170702	A	27-09-2001	AU 1607201 A	03-10-2001
			BR 0017157 A	26-11-2002
			CA 2402403 A1	27-09-2001
			CN 1450994 T	22-10-2003
			EP 1268438 A1	02-01-2003
			JP 2003528085 T	24-09-2003
			WO 0170702 A1	27-09-2001